

We claim:

1. A nucleic acid construct comprising inverted repeat sequences of a transposable element and an origin of transfer, wherein the origin of transfer lies between the inverted repeat sequences, such that a transposition event involving the inverted repeat sequences will result in the origin of transfer being included in the resultant insertion at the transposition target site.

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2. A construct according to claim 1, wherein the inverted repeat sequences are of, or derived from, the inverted repeat sequences of, a transposable element that employs a non-replicative transposition mechanism.

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3. A construct according to claim 1, wherein the inverted repeat sequences are, or are derived from, the OE and/or IE inverted repeat sequences of the transposon Tn5.

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4. A construct according to claim 3, wherein the inverted repeat sequences have at least 70% identity with any one or more of the following sequences:

a) 5'-CTGTC TCTTA TACAC ATCT-3'

3'-GACAG AGAAT ATGTG TAGA-5'

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b) 5'-CTGAC TCTTA TACAC AAGT-3'

3'-GACTG AGAAT ATGTG TTCA-5'

c) 5'-CTGTC TCTTG ATCAG ATCTT GATC-3'

3'-GACAG AGAAC TAGTC TAGAA CTAG-5'

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5. A construct according to claim 4, wherein the inverted repeat sequences have at least 85% identity any one or more of said sequences a), b) and c).

6. A construct according to claim 3, wherein the inverted repeat sequences have the sequence:

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5'-CTGTC TCTTA TACAC ATCT-3'
3'-GACAG AGAAT ATGTG TAGA-5'

7. A construct according to claim 1, which does not
5 encode a transposase.

8. A construct according to claim 1, wherein the origin
of transfer is an oriT which can be mobilised by the
helper plasmids pUZ8002 and pUB307.

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9. A construct according to claim 8, wherein the origin
of transfer has the sequence:

CCGGGCAGGA TAGGTGAAGT AGGCCCACCC GCGAGCGGGT GTTCCTTCTT
CACTGTCCCT TATTCGCACC TGGCGGTGCT CAACGGGAAT CCTGCTCTGC
15 GAGGCTGGC,

or a variant thereof having origin of transfer function.

10. A construct according to claim 1, which comprises a
promoterless reporter gene located between the inverted
20 repeat sequences.

11. A construct according to claim 10, wherein the
promoterless reporter gene is operatively associated with
a ribosome binding site and the construct further
25 comprises, upstream of the reporter gene and ribosome
binding site and between the inverted repeat sequences, a
translational stop sequence.

12. A construct according to claim 1, which lacks an
30 origin of replication.

13. A construct according to claim 1, which is linear and
consists essentially of the inverted repeat sequences and
any sequences located therebetween.

14. A vector including a construct according to claim 1.
15. A vector according to claim 14, which includes PCR
5 primer binding sites and/or restriction sites for the
amplification or excision from the vector of a linear
nucleic acid consisting essentially of the construct.
16. A method for mutagenising DNA of interest from a
10 bacterial species, the method comprising:
- a) contacting said DNA of interest with a nucleic
acid construct according to claim 1, to form a
transposition mixture;
 - b) incubating the transposition mixture under
15 conditions suitable for transposition to occur, said
contacting and incubating steps being performed other
than within cells of said bacterial species;
 - c) transferring transposed DNA of said
transposition mixture by conjugation from a donor
20 bacterial cell into a host bacterial cell; and
 - d) incubating the host cell under conditions
suitable for homologous recombination between the
transposed DNA and the DNA of the host cell.
- 25 17. A method according to claim 16, wherein the
transposition mixture also includes a transposase.
18. A method according to claim 17, wherein the
transposase is, or is derived from, Tn5 transposase.
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19. A method according to claim 18, wherein the
transposase is hyperactive mutant Tn5 transposase.

20. A method according to claim 16, wherein the DNA of interest is contained in one or more circular DNA molecules.

5 21. A method according to claim 16, wherein the construct is linear.

22. A method according to claim 16, wherein the contacting and incubating steps (a) and (b) occur outside
10 any bacterial cell, and the method comprises the further step (b1) of transferring the transposed DNA of the transposition mixture into the bacterial donor cell, prior to the conjugation step (c) into the host cell.

15 23. A method according to claim 16, which comprises an additional step, before the conjugation step, of identifying the site in the DNA of interest at which a transposition event has led to an insertion.

20 24. A method according to claim 16, wherein the DNA of interest is DNA from a bacterial library.

25 25. A method according to claim 24, wherein the DNA from which the library is generated is from bacteria of the genus *Streptomyces*.

26. A method according to claim 16, wherein the host cell is a pre-germinated spore.

30 27. A method according to claim 16, wherein the host cell is of the species or strain from which the DNA of interest originates.

28. A method according to claim 16, wherein the donor cell is of a different cell type from the host cell.
29. A method according to claim 16, which comprises an
5 additional step (e) of detecting whether homologous recombination has occurred in the host cell.
30. A method according to claim 29, wherein the
10 additional step (e) comprises: detecting the loss in the host cell of a selectable marker that is borne by the DNA of interest; and detecting the retention of a selectable marker that is borne by the construct.
31. A method according to claim 16, wherein the method
15 comprises, prior to the conjugation step, an additional step of replacing part or all of the transposition-derived insert by a further step of homologous recombination, to remove sequences from the insert and/or to add sequences to the insert.
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32. A method according to claim 16, which is carried out simultaneously on several DNA molecules of interest, which are conjugated from different donor cells into different host cells, to produce a plurality of
25 independently mutated host cells.
33. A method for mutagenising DNA of interest of a bacterial species, the method comprising the steps of:
- 30 a) contacting said DNA of interest with a nucleic acid construct according to claim 1, to form a transposition mixture;
- b) incubating the transposition mixture under conditions suitable for transposition to occur, said

contacting and incubating steps being performed other than within cells of said bacterial species; and

c) storing transposed DNA of said transposition mixture for future use.

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34. A method for mutagenising DNA of interest of a bacterial species, the method comprising, following the production and storage of transposed DNA according to claim 33 above, the steps of:

10 a) transferring said transposed DNA by conjugation from a donor bacterial cell into a host bacterial cell; and

b) incubating the host cell under conditions suitable for homologous recombination between the
15 transposed DNA and the DNA of the host cell.

35. A host cell producible or as produced by the method of claim 16.

20 36. A method of determining the effect of a genetic disruption, the method comprising culturing a host cell producible or as produced by the method of claim 16, and determining the effect of the disruption on the cell.

25 37. Transposed DNA of interest producible or as produced by the process according to claim 33.

38. Transposed DNA according to claim 37 contained in a bacterial cell or cells.

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39. A host cell producible or as produced by the method of claim 34.

40. A method of determining the effect of a genetic disruption, the method comprising culturing a host cell producible or as produced by the method of claim 34, and determining the effect of the disruption on the cell.